

Committee:
Jacobson, Chm.
Reimann
Lynch
Kotin

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street

#159R2

New York 17, N.Y.

Activated: 9/1/57

Renewed: 9/1/58*

(Cf. #74

Renewal
Application For Research Grant

Activated: 6/1/55

Renewed: 6/1/56

* (Anniversary date changed to
.1/1)

Date: Nov. 27, 1959

1. Name of Investigator: Cecilie Leuchtenberger, Ph.D.
2. Title: Senior Biologist and Cytochemist
3. Institution & Address: Children's Cancer Research Foundation
35 Binney Street
Boston, Mass.
4. Project or Subject: A correlated histological, cytological and
cytochemical study of the tracheobronchial tree from
mice exposed to cigarette smoke.

The interrelation between host factors and exposure
to cigarette smoke in the development of bronchial
lesions in mice.
5. Detailed Plan of Procedure:

A. SPECIFIC AIMS

The purpose of this project is the continuation and extension of the study of the sequence of events occurring in the tracheobronchial tree of mice exposed to cigarette smoke. Correlated histological, cytological and cytochemical investigations will be carried out and combined with the application of newer quantitative cytochemical techniques, such as microspectrophotometry and interference microscopy. It is felt that these methods, particularly when used simultaneously with other methods, such as fluorescence microscopy and tissue culture, will be of great help when attempting to explore the interrelationship between host factors and exposure to cigarette smoke in the development of bronchial lesions in mice.

Results of previous studies done in our laboratory on the project, which are concerned with the histopathological, cytopathological and cytochemical changes in the tracheobronchial tree and lungs of mice after exposure to cigarette smoke, have been reported at National and International Congresses and have been published under the titles:

I. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE TRACHEOBRONCHIAL TREE AND LUNGS OF MICE EXPOSED TO CIGARETTE SMOKE.

Bronchitis with Atypical Epithelial Changes in Mice

Exposed to Cigarette Smoke

Cancer, Vol. 11, No. 3, May-June, 1958

II. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE SEQUENCE OF EVENTS IN THE BRONCHIAL EPITHELIUM FROM MICE EXPOSED TO CIGARETTE SMOKE.

Acta Union Internationale contre le Cancer, Vol. XV, Nos. 3-4, 1959

III. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE TRACHEOBRONCHIAL TREE AND LUNGS OF MICE EXPOSED TO CIGARETTE SMOKE.

Varying Responses of Major Bronchi to Cigarette Smoke, Absence of Bronchogenic Carcinoma after Prolonged Exposure, and Disappearance of Bronchial Lesions after Cessation of Exposure.

Cancer (In press.)

IV. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE TRACHEOBRONCHIAL TREE AND LUNGS OF MICE EXPOSED TO CIGARETTE SMOKE.

Unaltered Incidence of Grossly Visible Adenomatous Lung Tumors in Female CF₁ Mice after Prolonged Exposure to Cigarette Smoke.

Cancer (In press.)

One of the main problems which seems to us to be of particular importance concerns the interrelation between host factors and exposure to cigarette smoke in the development of the bronchial lesions in mice. A thorough investigation of the possible role of the host factors is revealed by the findings of our previous studies as being both warranted and particularly pertinent. The data which are reported in the paper Varying Responses of Major Bronchi to Cigarette Smoke, Absence of Bronchogenic Carcinoma after Prolonged Exposure, and Disappearance of Bronchial Lesions after Cessation of Exposure disclosed that the variability in response of the major bronchi from mice after exposure to cigarette smoke is one of the most striking results of our studies. Differences in response were observed regardless of whether groups of mice were exposed to a few cigarettes for a short period or to many hundreds of cigarettes for nearly a lifespan. Since, in each group, all mice were handled in a standardized manner, host susceptibility must be taken into consideration when an attempt is made to interpret the relationship between changes in the major bronchi and exposure to cigarette smoke. While a differential response might be expected from mouse to mouse in a group of mice after exposure to smoke from a few cigarettes, it is indeed puzzling that such a wide spectrum of response, including resistance, persisted on chronic exposure. In some of the mice, even after exposure to over 1,000 cigarettes for nearly their whole lifespan, the bronchi were indistinguishable from those of nonexposed controls. In this connection it should be mentioned that the frequency of resistant cases increased considerably in the latter part of 1957 as compared with that encountered during the first phase of our study (1956). In 1958 we even observed a group of 45 mice without any lesions in the major bronchi, despite the fact that all 45 mice were exposed daily to smoke from three cigarettes for over three months. The increase in resistance was equally observed among groups of mice exposed to cigarette smoke in the smaller as well as in the 30% larger smoking chamber. In view of the fact that, to the best of our knowledge, all experimental conditions were kept the same in our laboratories during the years 1956-1958, we are unable to explain the change in incidence. However, since the mice were kept on a commercial diet which was repeatedly replenished (utilizing the same brand), changes in the composition of the food, including the addition of antibiotics and preservatives, cannot be excluded. One may also perhaps suggest that changes were made in the preparation of the cigarettes during the later periods of our experiments which may be responsible for the difference in the results. Whatever may develop as the cause for the increase of refractory cases, there can be no doubt that the differences in response, including complete resistance of the major bronchi to cigarette smoke, were encountered at all periods

1003541377

of our study. For example, among 23 cases presented in our first paper, six mice were found to have no alterations after exposure to cigarette smoke. The wide spectrum of findings and the independence of dose and duration of exposure to cigarette smoke would seem to detract from the importance of cigarette smoke as an injurious agent invariably affecting the major bronchi and would, rather, point to contributing factors characteristic for the individual animal. Actually, the difference in response of mice to cigarette smoke, including the considerable number of completely refractory cases, is not so surprising, but is in accordance with experience obtained in humans. Examination of the tracheobronchial tree of heavy smokers, as carried out by Auerbach and his associates, demonstrated a wide range of alterations, including cases without lesions. Furthermore, statistical data on the relationship between bronchogenic carcinoma and cigarette smoking have shown that, even among heavy smokers, only a relatively small percentage develop bronchogenic carcinoma.

Thus it appears that, in cases in which a harmful effect on the tracheobronchial tree has been observed after cigarette smoke, the cigarette smoke may not be solely responsible, but that there may be other elements within the host which may render the tissue susceptible to smoke. The concept that the tracheobronchial tree may, indeed, need a "special conditioning" for the injurious action of the tobacco smoke is supported by our data on mice - that the difference in response and resistance of the bronchi to cigarette smoke is independent of dose and length of exposure.

While it is impossible, at present, to relate any particular factor or factors of the host to the resistance or susceptibility of the tracheobronchial tree to cigarette smoke, the investigation of agents possibly carried by the host, which may contribute to the injury of the bronchi, would seem of great interest. Among the many possible host factors, viruses deserve special consideration - the more so since they occur with a certain frequency in mice and are capable of producing respiratory lesions.

It is well known that mice may be carriers of latent viruses which, under certain circumstances, may provoke alterations. In view of the increasing recognition of the dual role of viruses as either cytolytic agents or stimulators of cell proliferation, which may even lead to tumor formation, the investigation of viruses in combination with exposure to cigarette smoke seems to us particularly worthwhile. There are two main lines which we are investigating at present. The first is concerned with the detection of latent viruses in mice prior to exposure to cigarette smoke. It will be of interest to learn whether mice carrying a latent virus will exhibit a different response in the bronchi from that of mice free from virus. The second line is concerned with the effect of exposure to virus in addition to the exposure to cigarette smoke. If our concept is correct that viruses may act as co-factors in the production of bronchial lesions, then mice which carry latent viruses prior to exposure to smoke, or which are infected with viruses, should show frequent and perhaps more severe lesions, while mice free of virus should either be refractory or should show mild lesions.

B. Material and Methods: Essentially the same methods will be employed as in the previous studies. However, in addition to the microscopic, microspectrophotometric methods, special methods concerned with the detection and identification of viruses in mice after exposure to cigarette smoke, and after infection with respiratory viruses will be utilized, such as tissue culture and fluorescence microscopy. Attention will also be given to the possible role of nutritional factors.

6. Budget Plan:

Salaries	\$30,340.00
Expendable Supplies	1,300.00
Permanent Equipment	-
Overhead (15%)	4,821.00
Other (Travel)	500.00
Total	<u>\$36,961.00</u>

7. Anticipated Duration of Work:

Three to five years

8. Facilities and Staff Available:

The facilities which are available for these studies is an established laboratory of cytochemistry (Head, Dr. Cecilie Leuchtenberger) and suitable animal quarters in the Children's Cancer Research Foundation. The cytochemical laboratories are well-equipped for all tissue work, as well as for the analysis of intracellular substances. Some of the major items of permanent equipment are: (1) four microspectrophotometric apparatuses*; (2) one ultraviolet microspectrophotometer; (3) six research microscopes; (4) two phase microscopes; (5) two photomicrographic set ups; (6) one Beckman spectrophotometer; (7) one freeze drying apparatus for the fixation and embedding of tissues; (8) two interference microscopes; (9) tobacco research laboratory including newly designed and built smoking machine. There is also a team of workers (professional and technical) comprising cytochemist, pathologists, and research assistants.

* also fluorescence microscope.

9. Additional Requirements:

Personnel

1. Pathologist	\$13,000 + 3% Soc. Sec.	\$13,144
2. Experienced cytologist at Ph.D. level	6,000 + 3% " "	6,144
3. (2) research technicians trained in histological procedures and animal experimentation	7,200 + 3% " "	7,344
4. Part time secretary and research librarian	1,200 + 3% " "	1,236
5. Animal caretaker and glass washer	2,400 + 3% " "	<u>2,472</u>
Total Salaries and Social Security		\$30,340

1003541379

9. Additional Requirements (cont'd.):

Expendable Supplies

- | | |
|---|------------|
| 1. Animals and animal food
(approximate estimation) | \$500 |
| 2. Cigarettes and other laboratory
supplies (chemicals, glassware,
dyes, enzymes, etc.) | <u>800</u> |

Total expendable supplies \$1,300

10. Additional Information (Including relation of work to other projects and other sources of supply): - - -

/s./ Cecilie Leuchtenberger
Director of Project -
(Associate Professor of Cytology and Biology)
Senior Biologist and Cytochemist

/s./ Sidney Farber
President

1003541380